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Serum Vaccine Antibody Concentrations in Adolescents Exposed to Perfluorinated Compounds

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Running title: Vaccine antibodies in PFAS-exposed adolescents

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Abstract

Background: Postnatal exposure to perfluorinated alkylate substances (PFASs) is associated with lower serum concentrations of specific antibodies against certain childhood vaccines at age 7 years.

Objectives: We prospectively followed a Faroese birth cohort to determine these associations at age 13 years.

Methods: In 516 subjects (79% of eligible cohort members) aged 13 years, serum concentrations of PFASs and of antibodies against diphtheria and tetanus were measured and compared with data from the previous examination at age 7. Multiple regression analyses and structural equation models were applied to determine the association between postnatal PFAS exposures and antibody concentrations.

Results: Serum concentrations of PFASs and antibodies generally declined from age 7 to age 13. However, 68 subjects had visited the emergency room and likely received a vaccination booster, and a total of 202 children showed higher vaccine antibody concentrations at age 13 than at age 7. Separate analyses were therefore conducted after exclusion of these two subgroups. Diphtheria antibody concentrations decreased at elevated PFAS concentrations at ages 13 and 7 years, associations being statistically significant for PFDA at age 7 and PFOA at age 13, both suggesting a decrease by about 25% for each doubling of exposure. Structural equation models showed that a doubling in PFAS exposure at age 7 was associated with losses in diphtheria antibody concentrations at age 13 of 10-30% for the five PFASs. Few associations were observed for anti-tetanus concentrations.

Conclusions: These results are in accordance with previous findings of PFAS immunotoxicity at current exposure levels.

Introduction

Perfluorinated alkylate substances (PFASs) have a wide range of applications in water-, soil-, and stain-resistant coatings for clothing and other textiles, and oil-resistant coatings for food wrapping materials, and their uses for over 60 years have resulted in worldwide exposures to these persistent compounds (Lindstrom et al. 2011). Epidemiological research on possible adverse effects in exposed populations has intensified only in recent years (Grandjean and Clapp 2015; Steenland et al. 2014).

As a measure of depressed immune functions, antibody responses to routine childhood vaccinations has been identified as a sensitive indicator of elevated PFAS exposures in children (Grandjean et al. 2012; Granum et al. 2013). While pre-booster antibody concentrations at age 5 years appeared to be affected both by current and prenatal exposures (Grandjean et al. 2012), concentrations at age 7 showed inverse associations mainly with postnatal exposures (Grandjean et al. 2012; Mogensen et al. 2015). Decreased antibody responses to vaccinations have been reported in PFAS-exposed adults as well (Kielsen et al. 2015; Looker et al. 2014). In addition, elevated PFAS exposures seem to increase the risk of common infections in children (Granum et al. 2013). Prenatal exposures apparently did not affect childhood hospitalization rates (Fei et al. 2010), but the quality of the exposure assessment in this study has recently been called in doubt (Bach et al. 2015). As serum-PFAS concentrations are often highly correlated, epidemiological studies have not identified the PFASs mainly responsible, and some studies have therefore modeled total PFAS exposures (Grandjean et al. 2012; Mogensen et al. 2015). Results from *in vitro* studies of human leukocytes support the immunotoxic potential of several PFASs, including perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), and perfluorodecanoate (PFDA) (Corsini et al. 2012), and animal models suggest that immune depression may occur at serum concentrations of PFOA and PFOS similar to or slightly above those reported in exposed human populations

(DeWitt et al. 2012).

Immune functions are affected by many different kinds of stimuli (MacGillivray and Kollmann 2014), and specific antibody concentrations therefore vary between children with similar vaccination records, the main determinant of increased concentrations being recent booster vaccinations (Capua et al. 2013). While developmental exposure to polychlorinated biphenyls (PCBs) can also reduce vaccine responses (Heilmann et al. 2010), PCB concentrations were only weakly associated with the PFASs, and adjustment for prenatal and early postnatal PCB exposure did not materially affect the PFAS associations with antibody concentrations (Grandjean et al. 2012).

We have now extended up to age 13 years our follow-up of the Faroese birth cohort, where we most recently observed strong negative associations between postnatal serum-PFAS concentrations and antibody concentrations at age 7 years (Grandjean et al. 2012; Mogensen et al. 2015). We maintained our focus on tetanus and diphtheria, as these vaccines are toxoids that trigger complex immune system responses involving both T cells and B cells (Schatz et al. 1998). Our previous research showed that prenatal exposures were particularly linked to the pre-booster antibody concentration at age 5, while the concomitant exposure was associated with the response to the age-5 booster, and antibody concentrations at age 7 depended mainly on the current exposure levels (Grandjean et al. 2012; Mogensen et al. 2015). We therefore focused on the PFAS exposures reflected by serum concentrations from the two most recent examinations.

Methods

Study population

A birth cohort of 656 children was compiled from births at the National Hospital in Tórshavn in the Faroe Islands during 1997-2000 to explore childhood immune function and the impact

on vaccination efficacy (Grandjean et al. 2012). Faroese children receive vaccinations against diphtheria and tetanus at ages 3 months, 5 months, and 12 months, with a booster at 5 years, as part of the government-supported health care system. All children received the same amount of vaccines and associated alum adjuvant from the same source, although additional vaccines (pertussis and polio) were added to the booster during the project period (Grandjean et al. 2012). The study protocol was approved by the Faroese ethical review committee and by the institutional review board at Harvard T.H.Chan School of Public Health; written informed consent was obtained from all mothers.

All cohort members were invited for a follow-up examination at age 13 that included a physical examination and blood sampling. Information from the children's yellow vaccination record was copied, and the questionnaire on past medical history and current health status also included questions about vaccinations since the previous examination and whether the child had visited the emergency room. As tetanus vaccination (which includes the diphtheria toxoid) at the emergency room is a routine that may not have been recorded, hospital records did not contain further information on vaccinations that could be used for the purposes of the present study.

The PFAS concentrations were measured in serum obtained at the clinical examinations and frozen at minus 80 degrees shortly after separation. We used online solid-phase extraction and analysis using high-pressure liquid chromatography with tandem mass spectrometry (Grandjean et al. 2012). Within-batch and between-batch imprecision levels (assessed by the coefficient of variation) were about 5% or better for all analytes. Results with excellent accuracy have been obtained in regular comparisons organized by the German Society of Occupational Medicine. The PFASs quantified were perfluorohexanesulfonic acid (PFHxS), PFOA, PFOS, perfluorononanoate (PFNA), and PFDA.

Serum concentrations of major polychlorinated biphenyl congeners were

available from maternal pregnancy serum; the sum of the three major congeners was used at an indicator of the total PCB exposure as at previous occasions (Grandjean et al. 2012).

Serum concentrations of IgG antibodies were measured by the vaccine producer (Statens Serum Institut, Copenhagen, Denmark) using enzyme-linked immunosorbent assay for tetanus and, for diphtheria, a Vero cell-based neutralization assay using 2-fold dilutions of the serum. For both assays, calibration was performed using both international and local standard antitoxins. The methods were unchanged from previous examinations (Grandjean et al. 2012).

Statistical methods

Due to skewed distributions, antibody concentrations and serum-PFAS concentrations measured at ages 7 and 13 were all log-transformed (base 2) before they entered the models. Initial analyses were based on separate multiple linear regressions with an age-13 antibody concentration as the dependent variable and a serum-PFAS concentration as predictor along with age and sex as mandatory covariates, while also considering age-5 booster type (i.e., co-immunization with other vaccines (Grandjean et al. 2012)). We included adjustment for prenatal PCB exposure in separate analyses. We also carried out covariate adjusted logistic regression analyses to determine the odds ratios for each age-13 vaccine antibody concentration being below the clinically protective level of 0.1 IU/mL in regard to a two-fold change in PFAS exposures. Using structural equation models (Mogensen et al. 2015), we determined the associations of the age-7 PFAS concentrations with the specific antibody levels at age 13. We modeled these associations as an indirect effect (via the antibody result at age 7), and a total effect. The total effect has the same interpretation as a linear regression model without the adjustment for the antibody level at age 7 years, but provides a more stable measure with better error control. The indirect effect is the loss in antibody concentration at age 13 due to increases in PFAS at age 7 being associated with lower antibody concentrations

at that age, with these antibody concentrations being associated with the levels at age 13. In addition, in a separate model, we ignored the direct path from PFAS exposure at age 7 to the antibody concentration at age 13. All models were adjusted for sex and age as covariates, and incomplete observations were included assuming that information was missing at random, thus allowing calculations based on the maximum likelihood principle (Rubin 1987).

For comparison, we also applied linear mixed models to ascertain the effect of age-7 serum-PFAS concentrations on the antibody outcomes at ages 7 and 13. Interaction between exposure and age was included, and the results were again adjusted for both age and sex. The assumption of linear dose-response associations was verified by allowing for a more flexible relationship between the PFAS and the antibody concentration in generalized additive models using cubic regression splines with three knots (Hastie and Tibshirani 1990); no significant deviation from linearity was found. As both PFAS concentrations and antibody outcomes were log-transformed, we expressed the regression coefficients as the change in the antibody concentration in % for each doubling of the exposure.

Results

A total of 516 children (78.7% of cohort members) participated in the age-13 examinations, and 587 (89.0%) participated either at age 7 or at age 13, or both. The characteristics of the children who provided sufficient serum for analyses at age 13 are shown in Table 1. PFOS was by far the most prevalent PFAS with a median serum concentration at age 13 of 6.7 ng/mL, a 56% decrease since age 7. PFOA and PFNA decreased to a similar extent. While the correlation between the age-7 and age-13 concentrations of the same PFAS was even closer than the correlation between the different PFASs at each age (r up to 0.85 at age 13 and up to 0.62 at age 7 years), the close correlations prevented meaningful adjustment for concomitant PFAS exposures. In contrast, the PCB concentration in maternal pregnancy serum correlated

poorly with the child's serum-PFAS concentrations at age 7 (r between -0.06 and 0.15) and age 13 (r between 0.11 and 0.20).

On average, both antibody concentrations showed clear decreases during the six-year period from age 7 to age 13 years, although stronger for diphtheria (Table 1). At age 13, 207 (39.4%) children had antibody concentrations lower than 0.1 IU/mL for diphtheria and 103 (19.6%) for tetanus. However, not all children showed the anticipated decrease in antibody concentrations. Scatter plots of the correlation between antibody concentrations at ages 7 and 13 years show that concentrations increased between the two examinations in many cohort subjects (Figure 1). An increase in antibody concentration could likely be due to additional antigen exposure, most likely because some subjects had received an unscheduled booster dose. Thus, six of the children had received an additional booster at the project clinic because of their very low antibody concentrations after the age-5 booster. Questionnaire information further revealed that 68 cohort members had visited the emergency room where they likely received a tetanus booster shot, and many of them indeed had elevated antibody concentrations at age 13 (Figure 1). Still, a total of 202 children did not show the anticipated decrease in antibody concentrations between ages 7 and 13, most of whom not known to have been vaccinated after age 5. Statistical analyses were therefore carried out on the total group; after exclusion of subjects with a record of having visited the emergency room or otherwise having received an additional booster (no-ER group); and, for comparison purposes, after exclusion of all the 202 subjects who for unknown reason did not show the anticipated decrease in antibody concentrations between ages 7 and 13. These three groups were fairly similar in regard to sex, age, and PFAS exposures (Table 1).

Multiple regression analyses showed a uniformly inverse association between anti-diphtheria antibody concentrations at age 13 and the PFAS concentrations either at age 7 or age 13 years (Table 2). The tendencies were the strongest after exclusion of subjects with a

history of an additional booster, and an approximately 25% decrease for each doubling in the serum-PFOA concentration at age 13 and a 24% decrease for each doubling in PFDA at age 7 were both statistically significant. However, tetanus antibody concentrations, which had decreased much less than diphtheria antibodies, tended to show positive associations, one of them statistically significant (Table 3). As expected (Grandjean et al. 2012), adjustment for developmental PCB exposure had no appreciable effect on these associations, and PCB was therefore not further considered. Logistic regression analyses of the results, many of which were close to the 0.1 IU/mL limit, in most cases showed odds close to 1 for the antibody concentrations being below the clinically protective level (data not shown). However, for diphtheria, a two-fold increase in the concomitant serum-PFOA concentration showed odds ratios of 1.47 (95% CI: 1.03, 2.14; $p = 0.038$) and 1.71 (95% CI: 1.15, 2.55; $p = 0$ 0.008) for a non-protective antibody level in the total study group and in the no-ER group, respectively. Further, PFDA at age 7 showed statistically significant odds ratios of 1.39 (95% CI: 1.05, 1.85; $p = 0.023$) and 1.54 (95% CI: 1.13, 2.12; $p = 0.007$) for diphtheria in the same two groups; no other associations reached statistical significance.

In the first structural equation model, we included an indirect effect mediated by the exposure at age 7 via the antibody concentration at that age. Tables 4 and 5 show the indirect and the total effect observed in this model. For diphtheria, all associations were inverse, and all five PFASs showed statistically significant inverse associations in the no-ER group. Tendencies were weaker in the total group and after exclusion of subjects without decreasing antibody concentrations. For tetanus, some inverse associations were observed, though none of them significant. A second model without a direct effect fitted the data equally well and showed a similar, though stronger indirect effect for diphtheria via the age-7 antibody concentration. These tendencies also became clear for tetanus, where statistically significant indirect effects were now apparent in the no-ER group, both for PFOA (-24.2; 95%

CI: -41.1; -2.4) and for PFHxS (-25.1; 95% CI: -38.9; -8.3). Finally, the results obtained with the linear mixed models were similar to the first structural equation model.

Discussion

The present prospective study was carried out in adolescents to ascertain PFAS-associated decreases in antibody responses to their childhood vaccines. Due to numerous unscheduled booster vaccinations, the data were censored to remove those cohort members with a record of emergency room visit. In this subgroup, multiple regression results showed that diphtheria antibody concentrations decreased at elevated serum-PFAS concentrations at ages 13 and 7 years, associations being statistically significant for PFDA at age 7 and PFOA at age 13, both suggesting a decrease by about 25% for each doubling of exposure. The findings after exclusion of all subjects without a decrease in antibody concentrations showed less clear results. Structural equation models showed that a doubling in PFAS exposures at age 7 was associated with statistically significant losses in diphtheria antibody concentrations at age 13 of 10-30% for the five PFASs. Few associations were observed for anti-tetanus concentrations. However, more advanced modeling showed negative associations also for tetanus in regard to the age -7 PFAS exposure. Due to the inter-correlations between the serum-PFAS concentrations, further analysis of the possible role of individual PFASs was not pursued, and the associations observed may reflect the effects of the PFAS mixtures.

Although the present study aimed at obtaining prospective data on the associations between PFAS exposures and vaccine antibody concentrations over an extended period, an antibody concentration at a particular point in time does not represent the complete trajectory of changes and may not be representative of the protection against the specific disease in the long term. The present prospective study is apparently the first to elucidate the temporal changes in antibody changes in relation to PFAS immunotoxicity through to

adolescence. As a major obstacle in such observational studies, children and adolescents who visit the emergency room for cuts and other injuries are routinely administered a tetanus booster, thereby interfering with the study design, also affecting diphtheria, as both toxoids are present in the vaccine. We therefore chose to exclude subjects who had a record of an emergency room visit (no-ER group). For comparison, we carried out parallel calculations after exclusion of the more than 200 subjects who had not revealed the anticipated decrease in antibody concentrations between ages 7 and 13. With antibody concentrations that decrease to different degrees over time and with PFAS exposure levels that likewise show decreases, multiple regression analysis of the bivariate data sets need to be complemented by more advanced modeling.

Our study is limited to PFAS exposure assessments at two points during childhood at an interval of about six years. Although elimination half-lives of the PFASs of 2-5 years (Bartell et al. 2010; Olsen et al. 2007), the two widely separated measurements may not fully characterize the childhood exposure profile. As exposures during childhood are likely to vary (Kato et al. 2009; Lindstrom et al. 2011), it is possible that serial serum-PFAS analyses would provide stronger evidence for PFAS immunotoxicity. However, given that a decreased antibody concentration is most likely due to past, rather than the current, lower exposures (Mogensen et al. 2015), we chose to rely on the age-7 exposure levels and to include both antibody measurements at ages 7 and 13 in the assessment. At age 7, concurrent serum-PFAS concentrations showed strong, inverse associations with vaccine antibody concentrations, and inclusion of age-5 PFAS concentrations slightly strengthened these tendencies (Mogensen et al. 2015).

The 5-year booster vaccination is in principle the last booster vaccination that a child receives, and long-term protection is therefore intended. Already our previous results (Grandjean et al. 2012) showed that many children at age 7 had antibody concentrations

below the level assumed to provide the desired protection. This number had substantially increased by age 13 and was similar to the numbers below the level of protection at age 5 before the booster. These observations support the justification of routinely supplying a booster vaccination in emergency rooms in connection with any injury that may have involved the slightest tetanus risk. Unfortunately, such preventive measures add variance to the present study design, which relied on all children being vaccinated at the same age and with the same antigen dose. The study assumption was therefore violated, and it turned out to be difficult to adjust for additional immunizations at different ages, given the incomplete knowledge on booster administrations. Accordingly, the results clearly show the strongest associations between PFAS exposure and antibody concentrations in cohort subjects who were not known to have visited the emergency room or otherwise to have received a booster. However, Figure 1 also shows an increased antibody concentration at age 13 in many cohort members, possibly due to a booster that had not been recorded. Exclusion of all cohort members without an apparent decrease in the antibody concentration at age 13 may have more than remedied this problem, as it probably excluded too many subjects, thereby attenuating the statistical power. As could be expected, the results for the most restricted subgroup are somewhat weaker than the findings for the less restricted cohort. On the other hand, the increased antibody concentration in children who had received a booster suggest that any adverse influences of PFAS exposures could be remedied by repeated antigen challenge, although such intervention might not compensate for any other adverse effects associated with PFAS immunotoxicity.

As in previous studies (Grandjean et al. 2012; Mogensen et al. 2015), we found stronger associations for diphtheria than for tetanus. The former also exhibited much greater decreases from age 7 years to age 13, most likely because the diphtheria toxoid is a weaker antigen than tetanus and therefore more easily affected by PFAS-depressed immune functions.

The exact magnitude of the serum-antibody concentration may not be clinically important, but very low levels will result in poor or absent protection. With many antibody concentrations being close to the assumed clinically protective level of 0.1 IU/mL, logistic regressions showed only weak tendencies for antibody levels below the limit being associated with serum-PFAS concentrations. Likewise, imprecision of antibody assessments, especially at concentrations below or close to the clinically protective concentration, may have biased the regression analyses toward the null.

In support of our findings of PFAS immunotoxicity, a study of 99 Norwegian children at age 3 years found that the maternal serum PFOA concentrations were associated with decreased vaccine responses in the children, especially toward rubella vaccine, as well as increased frequencies of common cold and gastroenteritis (Granum et al. 2013). However, PFOS and PFOA concentrations in serum from 1400 pregnant women from the Danish National Birth Cohort were not associated with the total hospitalization rate for a variety of infectious diseases in 363 of the children up to an average age of 8 years (Fei et al. 2010). However, the validity of this study has been questioned by the poor stability of the serum-PFAS measurements (Bach et al. 2015); such imprecision of the exposure assessment could easily bias the results toward the null (Grandjean and Budtz-Jorgensen 2010).

In adults, PFOA exposure from contaminated drinking water was associated with lower serum concentrations of total IgA, IgE (females only), though not total IgG (C8 Science Panel 2009). More specifically, a reduced antibody titer rise after influenza vaccination was found in the most highly exposed subjects (Looker et al. 2014). Further, a small intervention study of 12 adults showed that the time-dependent increase in vaccine-specific antibody concentrations decreased at higher PFAS exposures (Kielsen et al. 2015). Thus, overall, support is building for the notion that PFAS exposure is associated with deficient immune functions. While diphtheria and perhaps tetanus may not be a likely hazard

in the Faroese and the residents of many other countries, the strongly decreased antibody concentrations likely reflect an immunological deficit. As optimal immune system function is crucial for health, the associations identified should be regarded as adverse. We recently calculated benchmark dose levels to estimate the magnitude of exposure limits that would protect against the immunotoxicity observed. The results suggested that current exposure limits may be 100-fold too high (Grandjean and Budtz-Jorgensen 2013). The present study extends the previous findings of deficient antibody responses in this cohort at younger ages and therefore adds support to the notion that substantially strengthened prevention of PFAS exposures is indicated.

Conclusions

The results are in accordance with previous findings of PFAS immunotoxicity at current exposure levels, although the results of this observational study are affected by concomitant decreases in concentrations of both PFASs and antibodies and by known and suspected booster vaccinations during the eight-year interval since the routine 5-year booster.

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Figure legend

Figure 1. Scatterplot showing paired antibody concentrations for diphtheria (left) and tetanus (right) in children examined at both ages 7 and 13 years.

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Table 1. Characteristics of children who contributed serum-antibody concentrations at the two follow-up examinations.

	Total cohort (N=587)		No ER visit (N=519)		No ER visits and no antibody increase (N=317)	
Variable	**N**	**Summary**	**N**	**Summary**	**N**	**Summary**
Sex, N(%), (Girls)	587 (100%)	278 (47.4%)	519 (100%)	238 (45.9%)	317 (100%)	147 (46.4%)
Booster type 1, N(%), (yes)	575 (98.0%)	412 (71.7%)	509 (98.1%)	367 (72.1%)	311 (98.1%)	209 (67.2%)
Age, median (IQR), (years)						
Current examination	516 (87.9%)	13.2 (12.9; 13.6)	448 (86.3%)	13.2 (12.9; 13.6)	317 (100%)	13.3 (13.0; 13.6)
Age-7 examination	565 (96.3%)	7.5 (7.5; 7.6)	497 (95.8%)	7.5 (7.5; 7.6)	317 (100%)	7.5 (7.5; 7.6)
Anti-body concentrations, median (IQR), (IU/mL)						
Anti-diphtheria at age 13 years	515 (87.7%)	0.1 (0.0; 0.2)	447 (86.1%)	0.1 (0.0; 0.2)	317 (100%)	0.1 (0.0; 0.2)
at age 7 years	459 (78.2%)	0.8 (0.4; 1.6)	391 (75.3%)	0.8 (0.4; 1.6)	317 (100%)	0.8 (0.4; 1.6)
Anti-tetanus at age 13 years	515 (87.7%)	0.6 (0.3; 1.8)	447 (86.1%)	0.5 (0.3; 1.2)	317 (100%)	0.5 (0.2; 1.0)
at age 7 years	459 (78.2%)	1.8 (0.6; 4.5)	391 (75.3%)	2.1 (1.1; 5.2)	317 (100%)	2.3 (1.3; 5.5)
PFAS concentrations, median (IQR), (ng/mL)						
PFOS at age 13 years	515 (87.7%)	6.7 (5.2; 8.5)	447 (86.1%)	6.7 (5.3; 8.5)	317 (100%)	6.8 (5.4; 8.7)
at age 7 years	488 (83.1%)	15.3 (12.4; 19.0)	420 (80.9%)	15.3 (12.4; 19.0)	312 (98.4%)	15.5 (12.9; 18.9)
PFOA at age 13 years	515 (87.7%)	2.0 (1.6; 2.5)	447 (86.1%)	2.0 (1.5; 2.5)	317 (100%)	2.0 (1.6; 2.6)
at age 7 years	488 (83.1%)	4.4 (3.5; 5.7)	420 (80.9%)	4.4 (3.6; 5.7)	312 (98.4%)	4.4 (3.5; 5.5)
PFHxS at age 13 years	515 (87.7%)	0.4 (0.3; 0.5)	447 (86.1%)	0.4 (0.3; 0.5)	317 (100%)	0.4 (0.3; 0.5)
at age 7 years	488 (83.1%)	0.5 (0.4; 0.7)	420 (80.9%)	0.5 (0.4; 0.7)	312 (98.4%)	0.5 (0.4; 0.7)
PFNA at age 13 years	515 (87.7%)	0.7 (0.6; 0.9)	447 (86.1%)	0.7 (0.6; 0.9)	317 (100%)	0.8 (0.6; 1.0)
at age 7 years	488 (83.1%)	1.1 (0.9; 1.5)	420 (80.9%)	1.1 (0.9; 1.5)	312 (98.4%)	1.1 (0.9; 1.5)
PFDA at age 13 years	515 (87.7%)	0.3 (0.2; 0.4)	447 (86.1%)	0.3 (0.2; 0.4)	317 (100%)	0.3 (0.2; 0.4)
at age 7 years	488 (83.1%)	0.4 (0.2; 0.6)	420 (80.9%)	0.4 (0.2; 0.5)	312 (98.4%)	0.4 (0.2; 0.5)

Abbreviation: IQR, inter-quartile range

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Table 2. Linear regression models of changes in anti-diphtheria concentrations at age 13 years associated with serum PFAS concentrations ages 13 and 7 years adjusted for sex, age at antibody assessment, and booster type. The change in the antibody concentration is expressed in % per doubling of the serum-PFAS concentration at the two different ages.

	Total cohort (N=587)				**No booster or ER visit (N=519)**				**No booster or ER visit and no antibody increase (N=317)**			
PFAS (ng/mL)	**N**	**Change**	**95% CI**	***p*-value**	**N**	**Change**	**95% CI**	***p*-value**	**N**	**Change**	**95% CI**	***p*-value**
PFAS concentrations age 13 years												
PFOS	505	-8.6	(-27.7; 15.6)	0.454	439	-10.5	(-29.8; 14.3)	0.374	311	-0.6	(-24.5; 30.9)	0.965
PFOA	505	-17.5	(-35.6; 5.8)	0.129	439	-25.3	(-42.5; -3.0)	0.029	311	-17.8	(-38.0; 9.0)	0.173
PFHxS	505	-5.5	(-22.9; 15.8)	0.583	439	-10.9	(-27.7; 9.8)	0.279	311	-0.2	(-20.4; 25.0)	0.984
PFNA	505	-4.5	(-24.2; 20.2)	0.693	439	-6.6	(-26.7; 19.0)	0.579	311	-3.7	(-25.8; 25.2)	0.780
PFDA	505	-3.7	(-22.0; 18.9)	0.726	439	-3.5	(-22.5; 20.3)	0.754	311	-4.4	(-24.9; 21.8)	0.716
PFAS concentrations age 7 years												
PFOS	427	-23.8	(-43.2; 2.3)	0.070	361	-25.6	(-45.4; 1.4)	0.061	306	-10.8	(-35.6; 23.5)	0.490
PFOA	427	-4.1	(-25.4; 23.3)	0.742	361	-9.2	(-30.7; 18.8)	0.480	306	-2.7	(-26.4; 28.5)	0.845
PFHxS	427	-10.2	(-25.7; 8.5)	0.264	361	-16.3	(-31.3; 2.0)	0.077	306	-5.9	(-23.4; 15.4)	0.556
PFNA	427	-11.3	(-27.4; 8.5)	0.243	361	-13.6	(-30.6; 7.5)	0.190	306	-7.0	(-25.6; 16.1)	0.519
PFDA	427	-21.5	(-34.4; -6.0)	0.008	361	-24.2	(-37.5; -8.0)	0.005	306	-19.7	(-34.0; -2.2)	0.029

%Change: Percentage change in antibody concentration per doubling of PFAS concentration

Table 3. Linear regression models of changes in anti-tetanus concentrations age 13 years associated with serum-PFAS concentrations age 13 and 7 years adjusted for sex, age at antibody assessment, and booster type. The change in the antibody concentration is expressed in % per doubling of the serum-PFAS concentration at the two different ages.

	Total cohort (N=587)				**No ER visit (N=519)**				**No ER visit and no antibody increase (N=317)**			
PFAS (ng/mL)	**N**	**Change**	**95% CI**	***p*-value**	**N**	**Change**	**95% CI**	***p*-value**	**N**	**Change**	**95% CI**	***p*-value**
PFAS concentrations age 13 years												
PFOS	505	22.2	(-12.4; 70.3)	0.237	439	23.4	(-7.0; 63.7)	0.144	311	14.8	(-8.7; 44.4)	0.236
PFOA	505	3.3	(-27.3; 46.9)	0.856	439	-5.6	(-30.5; 28.1)	0.710	311	-16.1	(-33.7; 6.3)	0.145
PFHxS	505	8.7	(-18.5; 45.0)	0.568	439	19.3	(-6.4; 52.1)	0.153	311	1.8	(-15.6; 22.9)	0.851
PFNA	505	15.2	(-16.9; 59.7)	0.394	439	5.1	(-20.7; 39.3)	0.727	311	11.6	(-10.3; 38.8)	0.324
PFDA	505	18.7	(-11.8; 59.8)	0.258	439	6.9	(-17.2; 38.0)	0.607	311	18.0	(-3.5; 44.4)	0.106
PFAS concentrations age 7 years												
PFOS	427	30.0	(-16.1; 101.4)	0.240	361	45.4	(1.2; 108.8)	0.043	306	2.7	(-21.8; 34.8)	0.849
PFOA	427	9.4	(-24.7; 58.9)	0.637	361	2.9	(-25.0; 41.1)	0.859	306	-4.9	(-24.6; 20.0)	0.671
PFHxS	427	14.8	(-13.3; 52.2)	0.334	361	25.2	(-0.6; 57.7)	0.057	306	-11.3	(-25.2; 5.2)	0.167
PFNA	427	31.0	(-2.7; 76.4)	0.075	361	23.1	(-4.6; 59.0)	0.110	306	11.9	(-7.1; 34.7)	0.235
PFDA	427	36.8	(4.7; 78.7)	0.022	361	25.1	(-0.4; 57.0)	0.054	306	3.5	(-12.3; 22.2)	0.682

%Change: Percentage change in antibodies per doubling of PFAS concentrations.

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Table 4. Effects of serum-PFAS concentrations age 7 years on anti-diphtheria antibody concentrations at ages 7 and 13 adjusted for age and sex in structural equation models. The change in the anti-diphtheria concentration is expressed in % per doubling of the age-7 PFAS concentration.

		Total cohort (N=587)			**No ER visit (N=519)**			**No ER visit and no antibody increase (N=317)**		
PFAS (ng/mL)	**Effect**	**Change**	**95% CI**	***p*-value**	**Change**	**95% CI**	***p*-value**	**Change**	**95% CI**	***p*-value**
PFOS	Indirect	-29.7	(-44.3; -11.3)	0.003	-32.8	(-47.9; -13.3)	0.002	-22.3	(-40.3; 1.2)	0.061
	Total	-26.5	(-45.7; -0.5)	0.046	-31.1	(-49.8; -5.4)	0.021	-16.0	(-38.8; 15.4)	0.282
PFOA	Indirect	-17.7	(-32.24; -0.0)	0.050	-19.8	(-35.4; -0.5)	0.045	-15.0	(-31.8; 5.9)	0.147
	Total	-4.3	(-26.0; 23.8)	0.739	-9.4	(-31.1; 19.2)	0.481	-5.8	(-27.8; 22.9)	0.661
PFHxS	Indirect	-13.4	(-25.9; 1.2)	0.071	-16.2	(-29.3; -0.6)	0.042	-8.6	(-22.6; 7.9)	0.289
	Total	-12.0	(-28.0; 7.5)	0.211	-19.5	(-34.7; -0.7)	0.043	-8.0	(-24.6; 12.4)	0.415
PFNA	Indirect	-20.7	(-31.8; -7.8)	0.003	-23.3	(-35.3; -9.0)	0.002	-18.1	(-31.6; -1.9)	0.030
	Total	-15.6	(-31.1; 3.2)	0.099	-17.4	(-33.7; 2.8)	0.087	-9.4	(-27.0; 12.5)	0.371
PFDA	Indirect	-19.6	(-28.8; -9.2)	<0.001	-20.7	(-30.7; -9.2)	0.001	-17.0	(-27.8; -4.7)	0.008
	Total	-22.5	(-34.5; -8.3)	0.003	-25.1	(-37.3; -10.4)	0.002	-19.8	(-32.6; -4.6)	0.013

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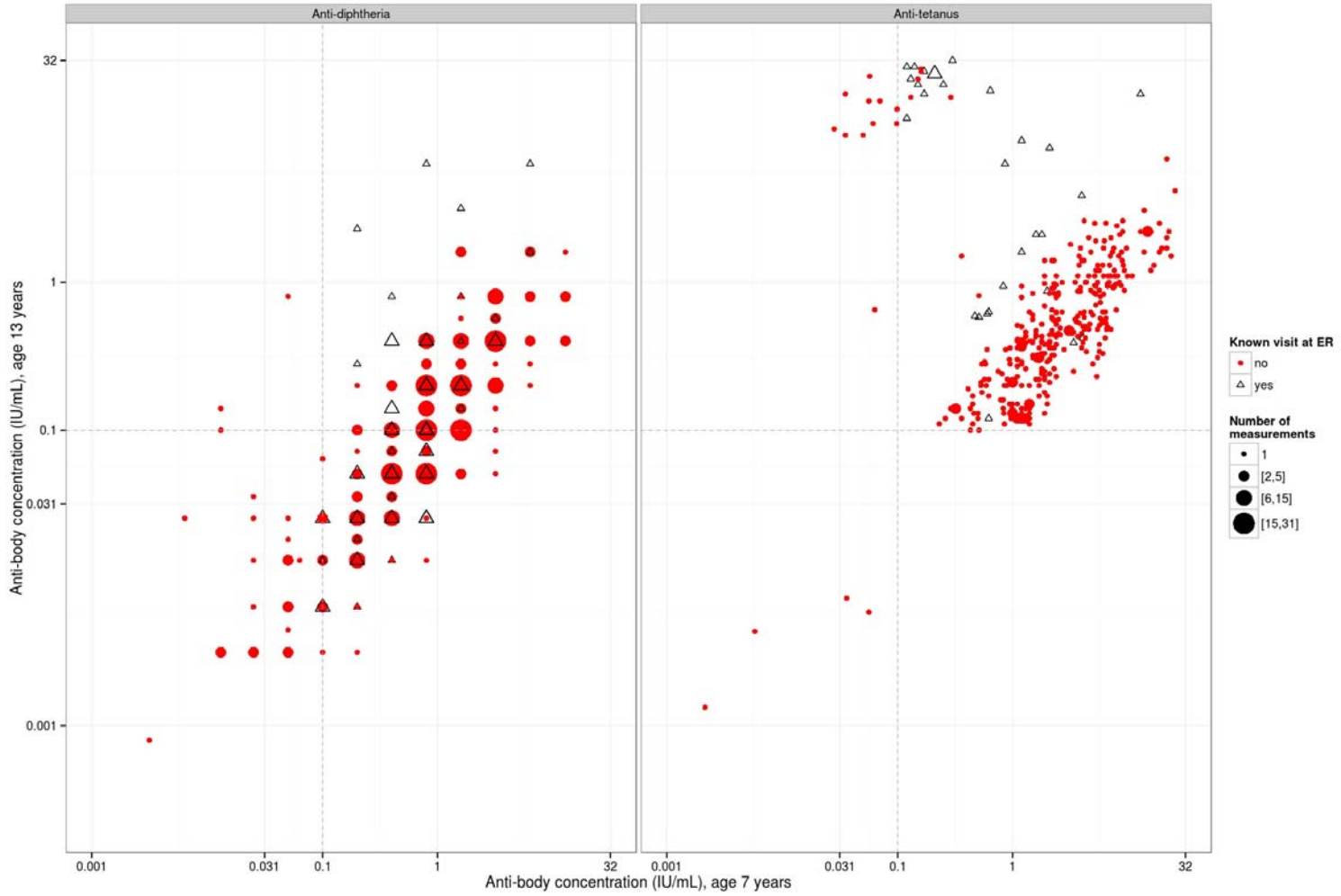
Table 5. Effects of serum-PFAS concentrations age 7 years on anti-tetanus antibody concentrations at ages 7 and 13 adjusted for age and sex in structural equation models. The change in the anti-tetanus concentration is expressed in % per doubling of the age-7 PFAS concentration.

		Total cohort (N=587)			**No ER visit (N=519)**			**No ER visit and no antibody increase (N=317)**		
PFAS (ng/mL)	**Effect**	**Change**	**95% CI**	***p*-value**	**Change**	**95% CI**	***p*-value**	**Change**	**95% CI**	***p*-value**
PFOS	Indirect	3.2	(-5.7; 12.9)	0.492	-2.2	(-6.5; 2.4)	0.347	-1.4	(-21.7; 24.2)	0.906
	Total	26.3	(-17.2; 92.6)	0.278	42.9	(-2.8; 110.0)	0.069	2.1	(-21.5; 32.9)	0.877
PFOA	Indirect	6.0	(-1.9; 14.4)	0.138	-3.2	(-7.7; 1.5)	0.181	-16.0	(-30.7; 1.8)	0.075
	Total	11.3	(-22.3; 59.3)	0.560	1.9	(-27.3; 42.6)	0.915	-7.2	(-25.5; 15.6)	0.505
PFHxS	Indirect	7.7	(0.6; 15.3)	0.033	-3.7	(-8.1; 0.8)	0.107	-12.4	(-24.2; 1.2)	0.072
	Total	14.1	(-13.1; 49.8)	0.342	25.0	(-2.3; 59.8)	0.075	-12.1	(-25.4; 3.7)	0.127
PFNA	Indirect	1.0	(-4.7; 6.9)	0.739	-1.1	(-3.8; 1.6)	0.416	4.2	(-10.9; 21.9)	0.604
	Total	28.9	(-3.1; 71.5)	0.081	23.6	(-6.0; 62.5)	0.129	12.7	(-5.8; 34.8)	0.190
PFDA	Indirect	-0.8	(-5.4; 4.0)	0.729	0.1	(-1.8; 2.0)	0.936	7.8	(-4.7; 21.8)	0.231
	Total	27.5	(-0.3; 62.9)	0.053	16.7	(-7.7; 47.5)	0.196	2.7	(-11.1; 18.5)	0.721

Figure 1.


Anti-diphtheria
Anti-tetanus
Anti-body concentration (IU/mL), age 13 years
Anti-body concentration (IU/mL), age 7 years
32
1
0.1
0.031
0.001
Known visit at ER
no
yes
Number of measurements
1
[2,5]
[6,15]
[15,31]